Title

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Journal

Genome announcements, 3(3)

ISSN

2169-8287

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Publication Date

2015-05-01

DOI

10.1128/genomea.00421-15

Peer reviewed

genomeA
Journals.ASM.org

Draft Genome Sequences of Four NDM-1-Producing *Klebsiella pneumoniae* Strains from a Health Care Facility in Northern California

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We report the draft genome sequences of *Klebsiella pneumoniae* strains from four patients at a northern California health care facility. All strains contained the New Delhi metallo-β-lactamase (NDM1) carbapenemase with extended antibiotic resistance, including resistance to expanded-spectrum cephalosporins, imipenem, ertapenem, and meropenem. NDM gene alignments revealed that the resistance was plasmid encoded.

Received 26 March 2015 **Accepted** 30 March 2015 **Published** 14 May 2015

Citation Greninger AL, Chorny I, Knowles S, Ng VL, Chaturvedi V. 2015. Draft genome sequences of four NDM-1-producing *Klebsiella pneumoniae* strains from a health care facility in northern California. Genome Announc 3(3):e00421-15. doi:10.1128/genomeA.00421-15.

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Klebsiella pneumoniae is a Gram-negative bacterium that is part of the normal flora of the gastrointestinal tract but can cause serious community- and hospital-acquired infections. Due to the recent evolution of multiple antibiotic resistance genes, *K. pneumoniae* has been denoted as a microbial threat to human health (1, 2). New Delhi metallo-β-lactamase (NDM1) carbapenemase-producing *K. pneumoniae*, originally reported from India, have spread rapidly around the world with many imported strains reported from California (3, 4).

Four *K. pneumoniae* strains came from adult males at a northern California health care facility in summer/fall 2013 and were derived from urine, sputum, a rectal swab, and pigtail drain fluid. The California Department of Public Health initially performed PCR testing for bla_{KPC} and bla_{NDM}.

All four strains were sequenced using Nextera paired-end sequencing and Nextera gel-free mate-pair sequencing and assembly with SPAdes v3.0/v3.1 and annotation via PROKKA v1.10 and NCBI Prokaryotic Annotation Pipeline (5, 6). The Nextera paired-end draft genome assemblies for strains CPH3020, CPH3707, CPH3823, and CPH5262 include 93, 116, 112, and 102 contigs >200 nucleotides measuring a total of 5,468,355 bp, 5,359,393 bp, 5,360,573 bp, 5,351,951 bp, respectively. The N_{50}s of the strains measure 214,475 bp, 206,548 bp, 206,548 bp, and 172,934 bp with coverages of 70×, 147×, 124×, and 155×, and there are 5,275, 5,182, 5,187, and 5,178 predicted coding sequences (CDS), respectively. Assemblies from the Nextera gel-free mate-pair alone yielded 68, 58, 54, and 59 contigs >200 nucleotides measuring a total of 5,494,465, 5,368,188, 5,371,617, and 5,378,024 bp, respectively. The mate-pair kit had significantly higher N_{50}s to 2,988,282 bp, 2,989,016 bp, 2,989,036 bp, and 2,989,036 bp with coverages of 65×, 48×, 45×, and 69×, respectively.

Of predicted CDS, 16.2% in strain CPH3020 were annotated as hypothetical proteins, while 15.2% were annotated as hypothetical proteins in the three other strains. GC content ranged from 57.1% for strain CPH3020 to 57.3% for the three other strains. The scaffolds for each genome demonstrated >99.9% nucleotide identity to each other and >99.5% nucleotide identity across >93% the bacterial chromosome of the reference genome *Klebsiella pneumoniae* MGH 78578. Less than 20% of the plasmid sequence of MGH 78578 was covered by scaffolds from these *Klebsiella* strains, with minimal contiguity. All four strains had bla_{NDM} resistance genes with 100% nucleotide identity to PMK1-NDM-1 and PittNDM01 NDM-1 genes, with the same flanking 10 kb, including the bleomycin resistance gene, phosphoribosyl anthranilate isomerase, Tn*3* family transposase, and GroES/L (7–9). All strains contained bla_{OXA-1} and bla_{SHV} and/or bla_{TEM} beta-lactamases (10, 11). None of the strains contained the bla_{KPC}, bla_{VIM}, or bla_{OXA-48} and/or $bla_{OXA-181}$ carbapenemase resistance genes nor the bla_{CTX-15} extended-spectrum beta-lactamase (12). The alignments of NDM and neighboring genes suggested that the resistance was plasmid encoded, with significant alignments spanning the entirety of the assembled contig to plasmids from NDM-containing strains of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter hormaechei*. Additionally, the NDM locus had 2× to 3× the coverage of the bacterial chromosome in three of the four strains, suggesting it existed on a different element. The additional ~100 kb in the CDPH3020 genome not present in the other *Klebsiella* strains was entirely derived from the pPMK1-NDM plasmid (9).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers JQCW00000000 (3020), JQCX00000000 (3707), JQDX00000000 (3823), and JQDY00000000 (5262) for the paired-end assemblies. The mate-pair assemblies have been deposited at DDBJ/EMBL/GenBank under the accession numbers

LAFV00000000 (3020), LAFX00000000 (3707), LAFW00000000 (3823), and LAFU00000000 (5262).

ACKNOWLEDGMENTS

We thank William Probert and Janet Ely for assistance with NDM-1 test and DNA isolation.

No external funding was received for this study.

REFERENCES

1. **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N.** 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis **10:**597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2.
2. **Boucher H, Talbot G, Bradley J, Edwards J, Gilbert D, Rice L, Scheld M, Spellberg B, Bartlett J.** 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **48:**1–12. http://dx.doi.org/10.1086/595011.
3. **Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, Savard P, Humphries RM, Kallen AJ, Limbago BM.** 2013. New Delhi metallo-β-lactamase producing *Enterobacteriaceae*, United States. Emerg Infect Dis **19:**870–878. http://dx.doi.org/10.3201/eid1906.121515.
4. **Rolain JM, Parola P, Cornaglia G.** 2010. New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect **16:**1699–1701. http://dx.doi.org/10.1111/j.1469-0691.2010.03385.x.
5. **Seemann T.** 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30:**2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.
6. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA.** 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **19:**455–477. http://dx.doi.org/10.1089/cmb.2012.0021.
7. **Dortet L, Nordmann P, Poirel L.** 2012. Association of the emerging carbapenemase NDM-1 with a bleomycin resistance protein in *Enterobacteriaceae* and *Acinetobacter baumannii*. Antimicrob Agents Chemother **56:**1693–1697. http://dx.doi.org/10.1128/AAC.05583-11.
8. **Doi Y, Hazen TH, Boitano M, Tsai Y-C, Clark TA, Korlach J, Rasko DA.** 2014. Whole-genome assembly of *Klebsiella pneumoniae* coproducing NDM-1 and OXA-232 carbapenemases using single-molecule, real-time sequencing. Antimicrob Agents Chemother **58:**5947–5953. http://dx.doi.org/10.1128/AAC.03180-14.
9. **Stoesser N, Giess A, Batty EM, Sheppard AE, Walker AS, Wilson DJ, Didelot X, Bashir A, Sebra R, Kasarskis A, Sthapit B, Shakya M, Kelly D, Pollard AJ, Peto TE, Crook DW, Donnelly P, Thorson S, Amatya P, Joshi S.** 2014. Genome sequencing of an extended series of NDM-producing *Klebsiella pneumoniae* isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. Antimicrob Agents Chemother **58:**7347–7357. http://dx.doi.org/10.1128/AAC.03900-14.
10. **Conlan S, Thomas PJ, Deming C, Park M, Lau AF, Dekker JP, Snitkin ES, Clark TA, Luong K, Song Y, Tsai Y-C, Boitano M, Dayal J, Brooks SY, Schmidt B, Young AC, Thomas JW, Bouffard GG, Blakesley RW, NISC Comparative Sequencing Program, Mullikin JC, Korlach J, Henderson DK, Frank KM, Palmore TN, Segre JA.** 2014. Single-molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing *Enterobacteriaceae*. Sci Transl Med **6:**254ra126. http://dx.doi.org/10.1126/scitranslmed.3009845.
11. **June CM, Vallier BC, Bonomo RA, Leonard DA, Powers RA.** 2014. Structural origins of oxacillinase specificity in class D β-lactamases. Antimicrob Agents Chemother **58:**333–341. http://dx.doi.org/10.1128/AAC.01483-13.
12. **Peirano G, Pillai DR, Pitondo-Silva A, Richardson D, Pitout JD.** 2011. The characteristics of NDM-producing *Klebsiella pneumoniae* from Canada. Diagn Microbiol Infect Dis **71:**106–109. http://dx.doi.org/10.1016/j.diagmicrobio.2011.06.013.